Potential Health Effects of Alkylphenols in Japan

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Abstract: More than twenty thousand tons of alkylphenols and alkylphenol ethoxylates are used annually in Japan in synthetic rubber industry, plastic, fabric, and metal processing industries as surfactants, cleaners, stabilizers for ethylcellulose, plasticizes, and phenol resins. Some forms of alkylphenols are reported to induce endocrine disruption especially in fish species. Data monitoring of inland aquatic environments conducted by the Ministry of Environment in 2000 reveal that the concentrations of nonylphenol in 71 monitoring sites (4.5% of all monitoring sites) exceeded the Predicted No Effect Concentration (PNEC) for fish. Even if there is no clear evidence of effects on human health by alkylphenols, the study revealed that the current environmental concentrations in these sites influence reproduction in fish species. As precautionary measures, it is recommended to curb emissions of alkylphenols and its ethoxylates into the aquatic environment.

Key words: Alkylphenol; Alkylphenol ethoxylate; Fish; Reproductive toxicity; Endocrine disruptors

Introduction

In 1991, Ana Soto *et al.* from the Tufts Medical School, Massachusetts, USA, observed an abnormal proliferation of MCF-7 breast tumor cells, grown without any stimulating agents in the culture medium. After a thorough investigation of the possible causes, they ascertained that the abnormal cell response was due to the polystyrene plastic tubes used in cell culture experiments.

Examining plasticware from various manufacturers led Soto *et al.* to the discovery that a chemical compound extractable from plasticware of a certain manufacturer is the source of contamination. Since the manufacturer refused to disclose the chemical, adducing "trade secret" reasons, identification of the compound

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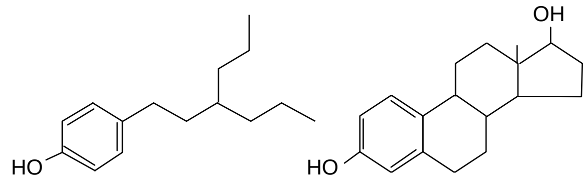
was not initially possible. Analysis of the chemical, performed in cooperation with specialists in the field, revealed that the component in the plastic was nonylphenol. Soto's group discovered that this synthetic chemical had estrogen-like activity and was responsible for proliferation of the breast tumor cells. Later, other estrogen-mimicking chemicals, including a metal can coating compound, bisphenol A, were also reported.

Sumpter *et al.* observed a high incidence of intersexuality (presence of both male and female gonadal features in the same animal) in roaches, a freshwater fish living in the River Lea in the South of Britain. To find the cause of this abnormal condition, they cultivated a number of rainbow trout in cages downstream from a number of sewage treatment plants, measured blood concentrations of vitellogenin (an egg-yolk protein) in male fish and concentrations of nonylphenol in the river water.

The results of their studies indicated that detergents containing alkylphenol ethoxylates used in wool processing factories, particularly those producing nonylphenol-group metabolites, are potentially the main cause of the intersex gonadal features observed in rainbow trout. They demonstrated a positive correlation between blood vitellogenin concentrations in male fish and nonylphenol concentration in water and reported that activated sewage sludge can degrade alkylphenol ethoxylates, resulting in the release of estrogenic alkylphenol metabolites into the water.

What are Alkylphenols?

The chemical structure of alkylphenols is based on the phenol ring with multi-carbon moieties: nonylphenol is a 9-carbon side chain alkylphenol; octylphenol has an 8-carbon alkyl chain; butylphenol has a 4-carbon side chain, and dodecylphenol is a 12-carbon side chain alkylphenol (Fig. 1). Nonylphenol and octylphenol are the most widely used alkylphenols and have the broadest range of application.



Nonylphenol
(one isomer with branched side chain)

β-estradiol

Fig. 1 Formula of alkylphenol

Alkylphenol production and consumption volumes decrease accordingly in the above mentioned order, the product with the smallest volume being dodecylphenol. Moreover, there are many alkylphenols with various chemical structures characterized by branched multi-carbon side chains.

Alkylphenols are mostly used as alkylphenol ethoxylates, that is, alkylphenols binding an ethoxy chain through their hydroxyl groups. The wide range of alkylphenol ethoxylate applications includes surfactants, ethylcellulose stabilizers, hydrophobic phenol resins, which are utilized in detergents, oil varnishes, synthetic rubber vulcanization accelerators, antioxidants of petroleum products and pesticide additives in agriculture.

Usage and Consumption Volume of Alkylphenol Ethoxylates

The annual alkylphenol ethoxylate production in Japan reached 46,850 t in 1998. As shown in Table 1, the largest discharge of alkylphenol metabolites is considered to be from fabrics, metal processing, and cleaning industries, among others; however, accurate data on the volume discharged into the environment in Japan is unavailable.

It has been estimated that distribution of alkylphenol compounds after release into the environment is 58–73% for water and 27–41% for estuarine sediments. Furthermore, alkylphenol compound levels in organs of animals high in the food chain hierarchy are not neces-

Table 1 Domestic Consumption of Alkylphenol Ethoxylates (as for 1998)

Synthetic rubber and plastic industry	4,200 (t)
Fabric industry	4,000
Metal processing industry	3,300
Business-use detergents	2,300
Cleaning industry	1,400
Dyes, pigments, paints, inks	1,100
Food processing industry	900
Agriculture	800
Paper and pulp industry	700
Petroleum and fuel industry	600
Civil engineering and construction industry	600
Pharmaceuticals and cosmetics industry	500
Leather auxiliaries	100
Others	3,400
Total consumption	23,900

sarily high and do not tend to get concentrated as a result of biological condensation, as demonstrated in the results of a study carried out by Tsuda *et al.* on fish species in Lake Biwa, Japan.

Alkylphenols in the Environment

In sewage treatment plants, alkylphenol ethoxylates are degraded aerobically with sequential cleavage of ethyl moieties and release of alkylphenols. According to the fact-finding survey performed by the Japan Environment Agency and Ministry of Construction in 1998/99, the alkylphenol concentrations in water samples ranged from ND (lower than or equal to the lowest detectable limit) to 21μg/l, with an average value of 0.22μg/l, and 0.59μg/l (95 percentile) was tentatively set as the estimated concentration for the environment, the highest alkylphenol compound concentration for an average aquatic locality.

Feminization of Fish Species

A committee at the Ministry of Environment has temporarily established 4 testing methods to examine the impact of endocrine disruptors on fish species. They are, as follows:

1. FLF/d-rR medaka test,
2. Reproduction test,
3. Vitellogenin assay, and
4. Medaka partial life cycle test.

The items 1., 3., and 4. are described below in more detail.

Regarding the FLF/d-rR medaka test and estrogen receptor binding activity, nonylphenol binds the estrogen receptor 10-times weaker than 17-β-estradiol (E2), in concentrations ranging from 10^{-9} to 10^{-8}M. Further, based on a reporter gene assay study with estrogen receptor-introduced cells, it was demonstrated that nonylphenol elicits transcription-stimulating activity hundreds times lower than E2.

On the other hand, in regard to the vitellogenin assay and alkylphenol concentrations affecting fish species, it was reported that an alkylphenol concentration capable of inducing abnormal microscopic changes in the testicular tissue of fathead minnow (a North-American cyprinid freshwater fish) was 1.6μg/l; in juvenile trout, the effective concentration that produced elevated liver vitellogenin levels was 10μg/l and in mature male trout, the concentration sufficient to induce elevated serum vitellogenin levels was 20μg/l.

Concerning medaka partial life cycle test, secondary sex characteristics changes (male fish feminization) were observed in male fish at alkylphenol water concentration of 23.5μg/l, with ovotestis and vitellogenesis at an alkylphenol water concentration of 11.6μg/l.

The medaka full life cycle test was performed in order to make a detailed examination of the alkylphenol effects on subsequent fish generations, revealed abnormal sex differentiation and lower fertilization rates among parental male fish at alkylphenol water concen-

tration of 17.7μg/l. In the second generation, the ovotestis condition was found to be present at the concentration of 8.2μg/l, a level not observed in the first generation males.

Ministry of Environment Survey —A Summary

The results of the survey on alkylphenols performed by the Ministry of Environment revealed that fish develop an ovotestis condition after exposure to low concentrations of octylphenol; which brings the endocrine disrupting activity of this alkylphenol compound into question. On the other hand, nonylphenol was considered a strong endocrine disruptor in fish, backed by results of *in vitro* studies on its nonylphenol ability to bind estrogen receptor and transcription-stimulating activity.

Based on the medaka partial life cycle test and alkylphenol concentration in water, the Lowest Observed Effect Concentration (LOEC) that affected fish sexual behavior was demonstrated to be 11.6μg/l, and the No Observed Effect Concentration (NOEC) to be 6.08μg/l. The Predicted No Effect Concentration (PNEC), the estimated concentration of alkylphenols in water that does not affect wildlife species, was calculated as 1/10 of NOEC value, that is 0.608μg/l.

Thus, with the PNEC being 0.608μg/l, water concentrations ranging from ND to 21μg/l in the domestic aquatic environment (data obtained from the environment fact-finding survey), and alkylphenol concentrations detected in 71 sampling locations (4.5%) exceeded the PNEC value. It was therefore considered that nonylphenol, in concentrations detectable in our country's aquatic environment, impacts on the endocrine system functions in fish species and possibly affects their reproductive system, as well.

Risk Reduction Measures

The advanced measures undertaken in many foreign countries to reduce alkylphenol ethoxylate applications, which target a number of industries, include various initiatives such as legislative regulations and usage limitations. In Japan, such actions include the ban on alkylphenol ethoxylate applications in domestic-use detergents and efforts to find alkylphenol substitutes for business- and industrial-use detergents. Hence, in implementing the Pollutant Release and Transfer Register system, it would be necessary to promote the automatic administration policy and cooperation between industry, the government, and academia.

Toxicity in Mammals

Although even very low alkylphenol concentrations produce harmful effects in fish species, alkylphenol toxicity in mammals has been demonstrated by acute oral toxicity test results in rats, the lethal dose being 50% (LD_{50}) of 1,200–2,400 mg/kg. Moreover, a repeated dosing study in rats revealed that pathological changes initially appear in the liver and kidneys. The no observable adverse effect (NOAEL) dose was found to be 100 mg/kg/day for non-branched nonylphenol and 50 mg/kg/day for branched nonylphenol.

Regarding the toxicity of alkylphenols on the reproductive system, on the second and third generation tests, endocrine disrupting activities such as weight increase of the uterus, weight decrease of the ovaries, decrease of sperm density in epididymides, elongation of the maturation period and other abnormalities were demonstrated with alkylphenols in doses of several 10 mg/kg/day. It has been considered that mammals are less sensitive to nonylphenol than fish. Similarly, *in vitro* studies have also demonstrated that both binding to the human estrogen receptor and transcription-stimulating activity in mammals are extremely weak in comparison with binding to the medaka estrogen receptor and transcription-stimulating activity in fish.

Estrogenic Activity of Alkylphenols

The effects of endocrine disruption by alkylphenols may be summarized as follows: 1. Added to the culture medium, alkylphenols stimulate *in vitro* proliferation of the MCF-7 breast tumor cells. 2. They bind to the estrogen receptor and elicit transcription-stimulating activity. 3. At the water concentration of 10 ppb, alkylphenols induce an increase of serum vitellogenin levels in male rainbow trout. 4. In mammalian species, they cause mammary gland cell proliferation and elongation of the sexual cycle, applied in 0.01 mg/kg dose in rat chronic oral toxicity test. 5. Moreover, male rats fed with 1 mg/*l* alkylphenol in drinking water in the prenatal period and from the 22nd day after birth, have lower-weight testis and lower sperm counts.

Thus, compared with fish species, mammals tend to be less sensitive to alkylphenolic compounds. One source of concern, however, is the low dose effect problem of endocrine disruptors.

Low Dose Effect of Endocrine Disruptors

Fred von Saal *et al.* from the University of Louisiana reported that treatment of pregnant rats with very low quantities of bisphenol A resulted in delivery of male offspring with lower prostate weights. The dose-response curve drawn in their experiment was not sigmoid-shaped, as is generally observed, but had an inverted U-shape. Furthermore, von Saal *et al.* also reported the early sexual maturity of female offspring born to rats treated with ethynyl estradiol during pregnancy. Their findings, known as the "low dose effects," have attracted considerable attention.

The reason for the special interest aroused by the "low dose effect" hypothesis is that until the publication of von Saal's results, toxic dose evaluation of a given pharmaceutical or chemical agent was based on the NOAEL calculation from the oscillating dose-response curve. Using the NOAEL value, a safety factor, NOAEL $\times 0.1$, was established with the specific difference between laboratory animals and humans defined as 0.1, and then taking into account the diverse patterns of human sensitivity to chemicals further multiplied by 0.1. The allowance dose was determined from the estimation that the safe dose equals NOAEL $\times$ 100-fold as the safety factor value.

If endocrine disruptors produce low dose effects, then this necessitates the redesigning of safety assessments for a whole range of chemicals, including subjects treated with even lower concentrations of a chemical or pharmaceutical agent into experimental groups, redoubling the cost burden as a result. The "low dose effect" hypothesis has already been openly and aggressively challenged by the chemical industry.

A similar phenomenon to low dose effects has been known to exist in biology; however, since the two arguments are at loggerheads, a conclusion has yet to be reached. However, the concept of reassessing safety doses for all chemical compounds taking the "low dose effect" into consideration does not appear to be the opinion of the majority.

Studies on Changes in Male Reproductive Functions

In 1987, Carlsen and Skakkebaek *et al.* issued a report based on a meta-analysis of papers published in various countries and related to semen qualities. Their findings indicated a significant decrease in sperm counts — by 50% over the past 50 years. The researchers proposed that the cause of such a condition was the prenatal disruption of reproductive organs development due to exposure to sex hormone-mimicking chemicals. Though their study drew some criticism concerning the differences between the subjects examined in the included papers, the inability to control the quality of data in some countries and the inappropriate statistical methods applied by some authors,

the work of Carlsen and Skakkebaek *et al.* made a major contribution to the research on male reproductive functions, which, since publication, has become more vigorous worldwide. Subsequently, reports on reproductive system abnormalities observed in wildlife species and the decline of semen quality in men have garnered widespread interest, leading to the establishment of fair-sized-budget research projects in Europe and America since the mid 1990s.

Likewise, in 1998, using what little basic data are available, we conducted research on the quality of semen among healthy Japanese men, in collaboration with the Department of Urology, School of Medicine, Sapporo University. In fact, the department mentioned above conducted research on healthy men from Sapporo some 20 years previously, and a comparison with the 1998 survey data revealed no marked changes in sperm count or sperm density. Since semen quality is influenced by periods of abstinence, sperm collection methods and daily fluctuations among individuals, the importance of a standardized system had been raised. An international quality control program led by Skakkebaek from Denmark, related to the research on male reproductive function has been highly promoted.

Additionally, we collected fat tissue from human autopsies to measure endocrine disruptor concentrations and to analyze sperm formation; an estimation of exposure to and impact from given endocrine disruptors was simultaneously conducted on the histopathological samples. During the study, our attention was drawn to lifelong changes in testes' weights. We discovered that, unlike general growth acceleration changes observed among young men from 1945 (the time of administrative autopsy program implementation), testes' weights did not follow the growth acceleration pattern of gradual increases in body height and weight, but entered a plateau phase or even decreased among men in their 20s born in and after 1970 (Fig. 2).

From the aforementioned observations and

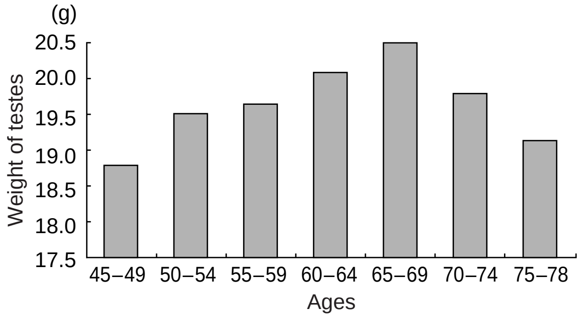


Fig. 2 Changes in weights of testes among men in their 20s

regarding risk assessment of the alkylphenol compounds, we concluded that since exposure to these endocrine disruptors in prenatal and neonatal period causes suppression of testis development, alkylphenols cannot continue to be considered safe for a mammalian organism. A more detailed and prospective survey is necessary.

Changes in Environmental Policies

In our country, the main objective and starting point for many environmental policies is to protect human health from any environmental pollulion. The Environment Agency has adopted specific measures to act sensibly in compensation cases for victims suffering from Minamata disease, Itai-itai disease, Yokkaichi asthma, and similar diseases. It is no exaggeration to say that to date, the environmental standards have predominantly been based on prevention of environmental harm to human health.

On the other hand, the US Environmental Protection Agency (US EPA), which aims to protect the survival of wildlife species, was established after the findings that exposure to DDT causes abnormal reproduction in birds. In fact, approval of a new pesticide in Europe and America requires reproductive toxicity testing in birds; this is not a preventive measure towards potentially harmful effects on the human reproductive system.

Regarding the effects of nonylphenol, the

Ministry of Environment has recognized the harmful effects of this alkylphenolic compound on reproduction in wildlife species and has established an environmental protection policy. Protection of wildlife species, which are more sensitive to environmental harm than humans, is an epoch-making decision.